1.07599.0025
1.07599.0100

Microscopy

Rhodamine B (C.I.45170)

for microscopy

IVD In Vitro Diagnostic Medical Device

CE

for fluorescence Tb staining of smears and sections

This staining dye “Rhodamine B (C.I.45170) - for microscopy” is used for human-medical cell diagnosis and serves the purpose of the bacteriological and histological investigation of sample material of human origin. It is a dry staining dye that is used to prepare a staining solution, that when used together with other in vitro diagnostic products from our portfolio makes target structures (e.g. acid-fast mycobacteria, by fixing, embedding, staining with the above rhodamine B solution, counterstaining, mounting) in bacteriological and histological specimen materials, for example smears of enriched bacterial cultures or histological sections of e.g. the lung, evaluable for diagnostic purposes.

Principle

Mycobacteria staining acc. to Truant, Brett and Thomas

The advantage of the fluorescence method is that the acid fast organisms appear bright fluorescence against a dark background and are easy to detect.

The cell wall of mycobacteria has a high proportion of wax and lipids and hence absorbs dyes only very slowly.
Once the mycobacteria have absorbed the fuchsin dye, it is virtually impossible to decolorize them again, even when they are intensively treated with a decolorizing solution such as e.g. hydrochloric acid in ethanol. Accordingly, mycobacteria are termed as acid- and alcohol-fast for staining.

Pretreatment of the specimens with Sputofluol® dissolves the bacteria from the surrounding viscid sputum and cell material. Sputofluol® also has a disinfectant effect, with the result that any microorganisms that are present are killed off.

Sample material

Smears of bacteriological material that have been air-dried, heat-fixed, and pretreated with Sputofluol® like sputum, smears from fine needle aspiration biopsies (FNAB), rinses, imprints, effusions, pus, exsudates, liquid and solid cultures
Sections of formalin fixed, paraffin embedded tissue (3 - 4 µm thick paraffin sections)

Reagents

Cat. No. 107599

Rhodamine B (C.I.45170) for microscopy — 25 g, 100 g

Color Index No.: 45170
Color Index Name: Basic violet 10

Also required:

Cat. No.	100206	Phenol GR for analysis ACS,Reag. Ph Eur	250 g, 1 kg
Cat. No.	100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	101301	Auramine O (C.I. 41000) for microscopy	50 g
Cat. No.	104095	Glycerol for fluorescence microscopy	250 ml
Cat. No.	105082	Potassium permanganate for analysis EMSURE® ACS,Reag. Ph Eur	250 g, 1 kg

Sample preparation

The sampling must be performed by qualified personnel.

Sputum

The mycobacteria should be pretreated with Sputofluol® to dissolve them from mucus and cellular structures. In this process, the active ingredient hypochlorite dissolves the organic material by oxidation and gently releases the mycobacteria so that they can be processed further.

Reagent preparation: Preparation of Sputofluol® solution 15 %

For preparation of approx. 100 ml solution mix:

Sputofluol®	15 ml
Distilled water	85 ml

Preparing sample material in centrifuge tubes:	
Sample	1 part (min. 2 ml)
Sputofluol® solution (15 % in distilled water)	3 parts
Shake vigorously	10 min
Centrifuge at 3000 - 4800 rpm	20 min
Decant supernatant Prepare smears of the sediment Air-dry	

Punctates, lavages, sediments

After appropriate enrichment measures, smear sample material on the slide and allow to air-dry.

Histological sections

Löffler’s methylene blue solution can be used to stain histological sections. Deparaffinize sections in the conventional manner and rehydrate in a descending alcohol series. Pretreatment with Sputofluol® is not necessary for specimens fixed with formalin.

Fixation

Specimens are fixed over a Bunsen burner flame (2 - 3 times, taking care to avoid excessive heating).
The specimens can also be fixed by heating at 100 - 110 °C in a drying cabinet or on a heating plate for 20 min.
Excessive temperatures or prolonged heating may involve a deterioration of the staining performance.

All samples must be treated using state-of-the-art technology.
All samples must be clearly labeled.
Suitable instruments must be used for taking samples and their preparation.
Follow the manufacturer’s instructions for application / use.

Reagent preparation

Liquified phenol

Phenol	
melt at 45 °C on a water bath	

Auramine O-rhodamine B solution

For preparation of approx. 250 ml solution mix:

Auramine O (C.I. 41000)	3 g
Rhodamine B (C.I.45170)	1.5 g
Glycerol	150 ml
dissolve at room temperature by stirring	
liquidified phenol (45 °C)	20 ml
add and mix	
Distilled water	100 ml
add and mix filter through glass wool	

The freshly prepared staining solution should be filtered before use.

Potassium permanganate solution

For preparation of approx. 100 ml solution mix:

Potassium permanganate	0.5 g
Distilled water	100 ml
dissolve	

Procedure

Staining on the staining rack

Deparaffinize histological slides in the conventional manner and rehydrate in a descending alcohol series.

The stated times should be adhered to to guarantee an optimal staining result.

Slide with fixed smear or histological sample		
Auramine O-rhodamine B solution	cover the slides, heat a) to 60 °C for 10 min or b) to 37 °C for 15 min	
Tap water	rinse carefully	
Hydrochloric acid in ethanol	differentiate until bleached	approx. 2 min
Tap water	rinse	
Potassium permanganate solution	cover	at least 2 min at most 4 min
Tap water	rinse	
Air-dry (e.g. over night or at 50 °C in the drying cabinet)*		

* Histological samples are not air-dried, after dehydration (ascending alcohol series) and clarification with xylene or Neo-Clear®, they can be mounted with water-free mounting agents (e.g. Neo-Mount®, Entellan®, DPX Neu, or Entellan® Neu) and a cover glass and can then be stored.

Covering with non-aqueous mounting media (e.g. Neo-Mount®, Entellan®, DPX new, or Entellan® new) and a cover glass is recommended for the storage of bacteriological specimens for several months. For this purpose, the stained specimens must be dried very well. When left unmounted, the stain remains stable for approx. 3 days, covered with immersion oil for just a few hours.

The use of immersion oil is recommended for the analysis of stained slides with a microscopic magnification >40x.

Result

Tb bacteria (AFB)	red or greenish fluorescence
Cells and mucus	dark

Evaluation

A positive result means “acid-fast rod bacteria present”, a negative result “acid-fast rod bacteria not present”. It is not possible to tell whether the bacteria found are *Mycobacterium tuberculosis* or of a different species of mycobacterium. The vitality (active, inactive) of the bacteria can also not be determined.
In the event that mycobacteria are detected, further examinations should be performed in specialized laboratories.

Trouble-shooting

Fixing

A sufficient degree of heat-fixing using a Bunsen burner or in a heating cabinet is essential to prevent the infectious potential of the specimens and further proliferation of the bacteria.

No staining of mycobacteria

The critical step of the mycobacteria-staining process is the decolorizing step, which can be influenced by the thickness of the specimen smear.
In addition, a freshly prepared solution of hydrochloric acid in ethanol is highly reactive, meaning that the result should be evaluated with caution. The incubation times stated in this protocol should be kept accurately in the decolorizing step, since otherwise false-negative results may ensue.

Technical notes

The microscope used should meet the requirements of a medical diagnostic laboratory.
When using automatic staining systems, please follow the instructions for use supplied by the supplier of the system and software.
The freshly prepared staining solution should be filtered before use.

Remove surplus immersion oil before filing.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel.
Valid nomenclatures must be used.
Further tests must be selected and implemented according to recognized methods.
Suitable controls should be conducted with each application in order to avoid an incorrect result.

Storage

Store Rhodamine B (C.I.45170) - for microscopy at +5 °C to +30 °C.

Shelf-life

Rhodamine B (C.I.45170) - for microscopy can be used until the stated expiry date.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at +5 °C to +30 °C.

The bottles must be kept tightly closed at all times.

Additional instructions

For professional use only.
In order to avoid errors, the application must be carried out by qualified personnel only.
National guidelines for work safety and quality assurance must be followed.
Microscopes equipped according to the standard must be used.
If necessary use a standard centrifuge suitable for medical diagnostic laboratory.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines.
Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines. Information on disposal can be obtained under the Quick Link “Hints for Disposal of Microscopy Products” at www.microscopy-products.com. Within the EU the currently applicable REGULATION (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, amending and repealing. Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006 applies.

Auxiliary reagents

Cat. No.	100206	Phenol GR for analysis ACS,Reag. Ph Eur	250 g, 1 kg
Cat. No.	100327	Hydrochloric acid in ethanol for microscopy	1 l, 5 l
Cat. No.	100579	DPX new non-aqueous mounting medium for microscopy	500 ml
Cat. No.	101301	Auramine O (C.I. 41000) for microscopy	50 g
Cat. No.	104095	Glycerol for fluorescence microscopy	250 ml
Cat. No.	104699	Immersion oil for microscopy	100-ml dropping bottle, 100ml, 500 ml
Cat. No.	105082	Potassium permanganate for analysis EMSURE® ACS,Reag. Ph Eur	250 g, 1 kg
Cat. No.	107960	Entellan® rapid mounting medium for microscopy	500 ml
Cat. No.	107961	Entellan® new rapid mounting medium for microscopy	100 ml, 500 ml, 1 l
Cat. No.	108000	Sputofluol® for microbiology and microscopy	1 l
Cat. No.	108298	Xylene (isomeric mixture) for histology	4 l
Cat. No.	109016	Neo-Mount® anhydrous mounting medium for microscopy	100-ml dropping bottle, 500 ml
Cat. No.	109843	Neo-Clear® (xylene substitute) for microscopy	5 l

Safety classification

Cat. No. 107599

Please observe the hazard classification printed on the label and the information given in the safety data sheet.
The safety data sheet is available on the website and on request.

Main components of the products

Cat. No. 107599

C.I. 45170 ≥ 90 %
$C_{28}H_{31}ClN_2O_3$
M = 479.02 g/mol

Other IVD products

Cat. No.	105174	Hematoxylin solution modified acc. to Gill III for microscopy	500 ml, 1 l, 2.5 l
Cat. No.	109093	TB-fluor Staining kit for fluorescence-microscopic detection of acid fast bacteria	6x 500 ml
Cat. No.	109204	Giemsa's azur eosin methylene blue solution for microscopy	100 ml, 500 ml, 1 l, 2.5 l
Cat. No.	109844	Eosin Y-solution 0.5% aqueous for microscopy	1 l, 2.5 l
Cat. No.	111609	Histosec® pastilles soldification point 56-58°C embedding agent for histology	1 kg, 10 kg (4x 2.5 kg), 25 kg
Cat. No.	111885	Gram-color stain set for the Gram staining method	1 set
Cat. No.	116450	Tb-color staining kit for the microscopic investigation of mycobacteria (cold staining)	1 set

Literature

1. Romeis - Mikroskopische Technik, Editors: Mulisch, Maria, Welsch, Ulrich, 2015, Springer-Verlag Berlin Heidelberg
2. Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 6th Edition
3. Theory and application of Microbiological Assay, Hewitt, W. and Vincent, S., 1989, Academic Press
4. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W. and Kiernan, J.A). Bios, 2002

Consult instructions for use

Manufacturer

Catalog number

Batch code

Caution, consult accompanying documents

Use by YYYY-MM-DD

Temperature limitation

Status: 2017-08-16

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